4 DNA ISOLATION

4.1 GOALS:

4.1.1 To develop a basic understanding of the theory and procedures of DNA isolation from blood stains, other biological stains, tissue, bone, teeth, hair and mixtures.

4.1.2 To become acquainted with the sensitivity of the isolation procedure.

4.1.3 To become familiar with the limitations of the isolation procedure.

4.1.4 To become familiar with the use of controls incorporated at this stage of the procedure.

4.1.5 To become familiar with the reagents used for DNA isolation and the function of each.

4.1.6 To become familiar with proper documentation of DNA isolation.

4.2 TASKS:

4.2.1 Prepare reagents necessary for DNA isolation.

4.2.2 Perform DNA isolation on at least 28 blood stains. Use the organic extraction method for 14 of the samples, the manual DNA IQ™ extraction method for 7 samples, and the automated DNA IQ™ extraction method (addressed in Chapter 6 of this manual) for the remaining 7 samples. Refer to the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedure Manual, Section III - Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 BIO System and Section IV - BioMek®2000 Automation Workstation Procedure Manual for the procedures. Initially use large stains (200 µl) and gradually move to smaller stains (5 µl) to test the ability to obtain DNA from smaller stains. These stains are prepared on a variety of different substrates commonly encountered in casework. Continue on to Chapter 5, DNA QUANTITATION - YIELD GEL and Chapter 7, DNA QUANTITATION – ALUQUANT® HUMAN QUANTITATION METHOD. Note: All 28 samples will be quantitated using the yield gel and the AluQuant® human quantitation methods to allow for a comparison of these quantitation techniques to be made. Complete the entire DNA analysis of these 28 samples before proceeding to task 4.2.3 below.

ATTENTION: Ensure that all appropriate controls are isolated with the training samples.

4.2.3 Perform DNA isolation on at least 28 other unmixed biological stains, including semen, vaginal fluid, saliva, vasectomized semen samples, urine and feces. These stains vary from large stains (200 µl) to smaller stains (5 µl) and are prepared on a variety of different substrates commonly encountered in casework. Use the organic extraction method for 14 of the samples and the manual DNA IQ™ extraction method for the remaining 14 samples. Refer to the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedure Manual, Section III - Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 BIO System for the procedures.

4.2.3.1 Extract semen stains (excluding vasectomized semen samples) using the "Organic/DNA IQ™ Extraction Method for Mixed Body Fluid Stains (Differential Procedure)" starting at the step which begins "Add to each sperm fraction .....".

4.2.3.2 Extract vaginal fluid, saliva, urine, and feces using the "Organic Extraction Method for Blood Stains and Tissue Samples or the DNA IQ™ Extraction Method for Buccal Cells, Blood Stains, Hairs, Envelopes, Stamps, and Cigarette Butts (Non-Differential Procedure)".

4.2.3.3 Extract vasectomized semen samples using the entire "Organic/DNA IQ™ Extraction Method for Mixed Body Fluid Stains (Differential Procedure)".

4.2.3.4 Continue on to Chapter 7, DNA QUANTITATION – ALUQUANT® HUMAN QUANTITATION METHOD. Complete the entire DNA analysis of these 28 samples before proceeding to task 4.2.4 below.

ATTENTION: Ensure that all appropriate controls are isolated with the training samples.

4.2.4 Perform DNA isolation on at least 20 mixed biological stains. Each stain will consist of a mixture of two biological fluids, to include semen, vaginal fluid, blood or saliva. Use the manual differential extraction methods found in the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedure Manual, Section III - Fluorescent Detection PCR-Based STR DNA Protocol: PowerPlex® 16 BIO System. Ten of the mixed body fluid stains should be extracted using the organic differential extraction method. Five of the mixed body fluid stains should be extracted using the manual DNA IQ™ extraction method and the remaining 5 mixed body fluid stains should be extracted using the automated DNA IQ™ extraction method addressed in Chapter 6 of this manual and outlined in the Commonwealth of Virginia Department of Forensic Science Forensic Biology Section Procedure Manual, Section IV - BioMek®2000 Automation Workstation Procedure Manual. As above, stains are prepared on a variety of different substrates commonly encountered in casework and vary in size and quantity of fluid present. Continue on to Chapter 7, DNA QUANTITATION – ALUQUANT® HUMAN QUANTITATION METHOD. Complete the entire DNA analysis of these 40 samples before proceeding to task 4.2.5 below.

ATTENTION: Ensure that all appropriate controls are isolated with the training samples.

4.2.5 Perform DNA isolation on the following validation samples using either an organic extraction method or the DNA IQ™ extraction method (manual or robotic).

4.2.5.1 Two blood/bone/tissue sample sets, each set from a different individual and each set containing 1 blood sample, 2 bone samples, and 4 tissue samples (total of 14 samples - 2 blood, 4 bone, and 8 tissue samples)

4.2.5.2 Five animal samples

4.2.5.3 Ten contaminated stains (contaminants such as bacteria, soil, grass, cleaning agents, etc.)

4.2.5.4 Five blood/semen/hair sample sets, each set from a different individual (total of 15 samples - 5 blood, 5 semen, and 5 hair samples)

4.2.5.5 A family study (at least 5 samples)

4.2.5.6 Non-probative case samples (at least 5 cases)

4.2.5.7 Old stains - at least 5 years old (10 samples)

4.2.5.8 Two buccal swab/teeth sample sets, each set from a different individual (total of 4 samples - 2 buccal swabs and 2 teeth)

4.2.5.9 Continue on to Chapter 7, DNA QUANTITATION – ALUQUANT® HUMAN QUANTITATION METHOD.

ATTENTION: Ensure that all appropriate controls are isolated with the training samples.

4.2.5 Read applicable literature and become familiar with the glossary terms. Refer to Appendices A, B, and C.

4.3 TRAINING EVALUATION:

4.3.1 Knowledge

4.3.1.1 Review of notes and worksheets in training notebook by training coordinator.

4.3.1.2 Mini -mock trials/oral and practical examinations.

4.3.2 Skills

4.3.2.1 The trainee should perform DNA isolation on a sufficient variety and number of samples to develop and exhibit an unquestionably sound technique for successfully isolating DNA. This will be monitored by review of the documentation in the training notebook and continual observation by the training coordinator.

4.3.3 Completion of the trainee checklist by the training coordinator.

STUDY QUESTIONS:

1. Using the following methods, how do you extract DNA from a blood sample? a semen sample?
   a. Organically
   b. DNA IQ™ extraction method (manual and robotic)
2. What is stain extraction buffer and how does it work? DNA IQ™ Lysis Buffer?
3. What is TNE? When is it used and how does it work?
4. What is the function of SDS in the isolation procedure?

5. What is the function of Proteinase K in the isolation procedure?

6. What is the function of Sarkosyl in the isolation procedure?

7. What is the function of the organic extraction portion of the procedure? What is the function of phenol, chloroform, and isoamyl alcohol in the extraction procedure?

8. Explain the use of DTT in isolation.

9. What are histones? protamines?

10. Why is it important to autoclave reagents and certain supplies?

11. Explain the use of Microcon filters.

12. Why is the chelex extraction method not used by the DFS?

13. Does the DNA IQ™ System isolate only human genomic DNA? Please explain your answer.

14. Is the DNA obtained using the DNA IQ™ System single-stranded or double stranded? Why?

15. What is the purpose for heating the DNA sample once the DNA IQ™ Elution Buffer has been added to the sample?

16. What may be some of the reasons why inconsistent DNA yields may be obtained with the DNA IQ™ System?

17. Which method is more efficient in isolating DNA from samples containing a low level of DNA versus a higher level of DNA and why?

CHECKLIST FOR DNA ISOLATION

Name of Trainee: _____

1. Preparation of reagents necessary for DNA isolation.

   Date:_____ Training Coordinator:_____

   Comments:_____

2. Trainee has successfully and accurately completed all appropriate paperwork associated with the DNA extraction process.

   Date:_____ Training Coordinator:_____

   Comments:_____

3. Trainee has successfully isolated DNA:

   Organically extracted DNA from a minimum of 14 blood stains on different substrates and stains ranging in size (5 µL to 200 µL).

   Date:_____ Training Coordinator:_____

   Comments:_____

   Using the manual DNA IQ™ extraction method isolated DNA from a minimum of 7 blood stains on different substrates and stains ranging in size (5 µL to 200 µL).

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   Date:_____ Training Coordinator:_____

   Comments:_____

   Using the automated DNA IQ™ extraction method isolated DNA from a minimum of 7 blood stains on different substrates and stains ranging in size (5 µL to 200 µL).

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   Date:_____ Training Coordinator:_____

   Comments:_____

   Estimated the amount of isolated DNA from all 28 blood stains using a yield gel and the AluQuant® human quantiation methods.

Date:_____ Training Coordinator:_____

Comments:_____

Organically extracted DNA from a minimum of 14 unmixed biological stains, to include semen, vaginal fluid, saliva, vasectomized semen, urine, and feces on different substrates and stains ranging in size (5 µL to 200 µL).

Date:_____ Training Coordinator:_____

Comments:_____

Successfully isolated DNA from a minimum of 14 unmixed biological stains, to include semen, vaginal fluid, saliva, vasectomized semen, urine, and feces on different substrates and stains ranging in size (5 µL to 200 µL) using the DNA IQ™ extraction method.

Date:_____ Training Coordinator:_____

Comments:_____

Estimated the amount of isolated DNA from all 28 unmixed biological stains using the AluQuant® human quantiation method.

Date:_____ Training Coordinator:_____

Comments:_____

Extracted DNA, using the organic differential method, for a minimum of 10 mixed biological stains, to include semen, vaginal fluid, blood, and saliva.

Date:_____ Training Coordinator:_____

Comments:_____

Extracted DNA, using the manual DNA IQ™ differential method, for a minimum of 5 mixed biological stains, to include semen, vaginal fluid, blood, and saliva.

Date:_____ Training Coordinator:_____

Comments:_____

Extracted DNA, using the automated DNA IQ™ differential method, for a minimum of 5 mixed biological stains, to include semen, vaginal fluid, blood, and saliva.

Date:_____ Training Coordinator:_____

Comments:_____

Estimated the amount of isolated DNA from all 20 mixed biological stains (this should include the sperm and non-sperm fractions for each mixed sample, where appropriate) using the AluQuant® human quantiation method.

Date:_____ Training Coordinator:_____

Comments:_____

Performed DNA isolation on the following validation samples using either an organic extraction method, manual DNA IQ™ extraction method or the automated DNA IQ™ extraction method.

- Two blood/bone/tissue sample sets, each set from a different individual
- Five animal samples
- Ten contamination stains
- Five blood/semen/hair sets
- A family study
- Non-probative case samples (at least 5 cases)
- Ten old stains (at least 5 years old)
- Two buccal swab/teeth sample sets

Date:_____ Training Coordinator:_____

Comments:_____

Estimated the amount of isolated DNA from all validation samples using the AluQuant® human quantiation method.

Date:_____ Training Coordinator:_____

Comments:_____

4. Trainee has developed a thorough understanding of the organic, manual DNA IQ™ and automated DNA IQ™ extraction methods, to include differential extractions.

Date:_____ Training Coordinator:_____

Comments:_____

5. Notebook is organized and complete.

   Date:_____ Training Coordinator:_____

   Comments:_____

6. Trainee has read and understands all applicable literature.

   Date:_____ Training Coordinator:_____

   Comments:_____

7. Trainee has participated in mini-mock trials and/or question and answer sessions.

   Date:_____ Training Coordinator:_____

   Comments:_____

♦END